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ORIGINAL ARTICLE

Computer-aided molecular design of (E)-N-Aryl-2-ethene-sulfonamide analogues as microtubule targeted agents in prostate cancer

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KEYWORDS

3D-QSAR;
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ADMET

Abstract Microtubules are tube-shaped, filamentous and cytoskeletal proteins that are essential in all eukaryotic cells. Microtubule is an attractive and promising target for anticancer agents. In this study, three-dimensional quantitative structure activity relationships (3D-QSAR) including comparative molecular field analysis, CoMFA, and comparative molecular similarity indices analysis, CoMSIA, were performed on a set of 45 (E)-N-Aryl-2-ethene-sulfonamide analogues as microtubule-targeted anti-prostate cancer agents. Automated grid potential analysis, AutoGPA module in Molecular Operating Environment 2009.10 (MOE) as a new 3D-QSAR approach with the pharmacophore-based alignment was carried out on the same dataset. AutoGPA-based 3D-QSAR model yielded better prediction parameters than CoMFA and CoMSIA. Based on the contour maps generated from the models, some key features were identified in (E)-N-Aryl-2-arylethene-sulfonamide analogues that were responsible for the anti-cancer activity. Virtual screening was performed based on pharmacophore modeling and molecular docking to identify the new inhibitors from ZINC database. Seven top ranked compounds were found based on Gold score fitness function. *In silico* ADMET studies were performed on compounds retrieved from virtual screening in compliance with the standard ranges.

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1. Introduction

Microtubules made up of α - and β -tubulin heterodimers, are dynamic, long and filamentous protein polymers (Fig. 1a). They are vital in the growth and preservation of cell shape (Jordan and Wilson, 2004) and have key roles in migration, signaling and cell propagation in eukaryotic cells. Microtubules are very crucial in the process of mitosis in stage followed by equal division of duplicated chromosomes before

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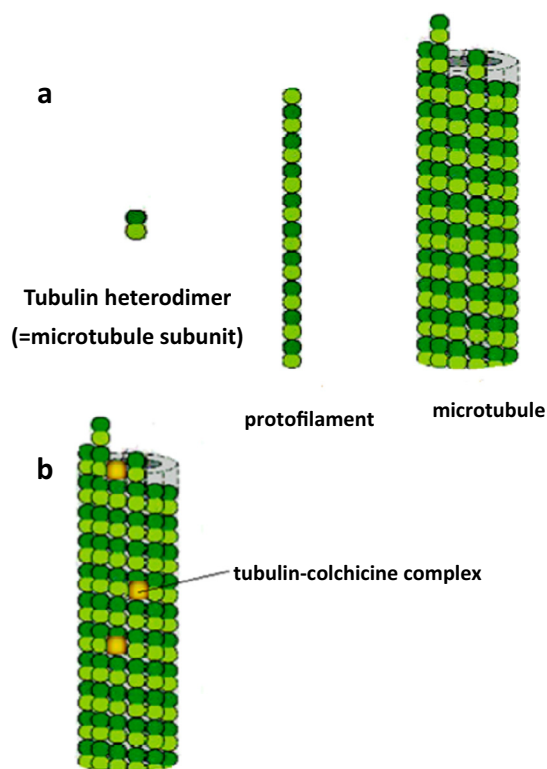


Figure 1 (a) The subunit of each protofilament is tubulin heterodimer which is created from a pair of α - and β -tubulin monomers. Protofilaments arrange in parallel to form microtubule. (b) Colchicine represses microtubule dynamics by complex formation with tubulin heterodimers and copolymerization into microtubule.

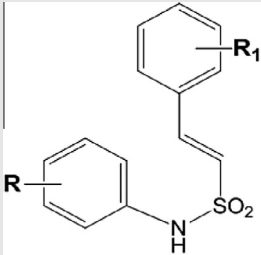
conversion of the cell into two daughter cells. Dynamic structures and role of microtubules in mitosis make them become an important target for anticancer drugs. Microtubule binders have been considered as a member of pharmacopoeia of cancer for decades. However, before the advent of targeted therapy microtubules, DNA was only cancer therapeutic target (Dumontet and Jordan, 2010). Microtubule binding agents are divided into two major classes according to the mechanism of amendment of microtubule dynamics: agents that inhibit microtubule polymerization and agents that enhance its polymerization. Both classes cause cell cycle stop at G2/M and finally cell death (Amos, 2011; Chou et al., 1994; Jordan and Wilson, 1998, 2004; Panda et al., 1996; Wilson et al., 1999). Colchicine is one of the most important inhibitors of tubulin polymerization but its toxicity in clinical trials has led to not finding wide application in therapy of cancer (Fig. 1b). So, there is an essential requirement to propose new inhibitors for microtubules (Hearn et al., 2007; Pellegrini and Budman, 2005). Many molecules for inhibition of polymerization which compete with site of colchicine binding of tubulin have been introduced so far. But until now, no colchicine-site binder has been authorized for therapy of cancer (Chaplin et al., 2006; Hsieh et al., 2005; Kemnitzer et al., 2009; Lee et al., 2010; Mahindroo et al., 2006; Nam, 2003; Pirali et al., 2006; Sirisoma et al., 2009; Tron et al., 2005; Xie et al., 2011; Zhang et al., 2007).

Quantitative structure–activity relationship (QSAR) has a fundamental role in computer-assisted-drug discovery. This method tries to determine a reliable relationship between molecular attributes and biological activity (Du et al., 2008a,b, 2005a, 2008b, 2009b; Prado-Prado et al., 2008; Wei et al., 2009). So far, many different QSAR studies have been accomplished for discovery of anti-cancer agents, including anti-prostate agents (Marzaro et al., 2011; Munteanu et al., 2009; Planche et al., 2013; Prakash and Khan, 2013; Speck-Planche et al., 2011a,b, 2012a,b; Vilar et al., 2009). 3D-QSAR, namely CoMFA (Cramer et al., 1988b) and CoMSIA (Klebe and Mietzner, 1994) uses three-dimensional shape of molecules and serves as useful guide for the design and generation of new drugs with more bioactivity or selectivity. The grid potential analysis employing in these models creates spatial distribution of effective fields as independent variables about superimposed molecules (Cramer et al., 1988b). The spatial distribution extremely depends on bioactivity of conformation of active ligands. In cases that 3D structure of bioactive conformations is not available, generation of a valid 3D-QSAR model for the exhibition of actual active site of receptor is difficult (Asakawa et al., 2012). In AutoGPA modeling (Meena et al., 2011), pharmacophore features that are common among most active ligands are used to find bioactive conformers of ligands and align them. According to the definition by IUPAC, "a pharmacophore is an ensemble of steric and electronic features that is necessary to ensure the optimal supramolecular interactions with a specific biological target and to trigger (or block) its biological response" (Wermuth et al., 1998). In the present study, we developed pharmacophore-based AutoGPA model to identify the critical pharmacophore features necessary for potent inhibitors. The popular CoMFA and CoMSIA models were built in comparison with AutoGPA model on a set of (E)-N-Aryl-2-ethene-sulfonamide analogues as anti-prostate cancer agents (Ramana Reddy et al., 2013). At present, Prostate cancer is second reason of mortality due to cancer in men in the United States (Jemal et al., 2010). Recently, many investigations were done to treat this cancer (Cai et al., 2012). In order to investigate interaction of tubulin and these analogues, molecular docking was applied. Molecular docking studies (Du et al., 2009a; Huang et al., 2008) can provide useful information for in-depth understanding of some subtle action mechanisms at the molecular biology level, such as the marvelous allosteric mechanism revealed recently by the NMR observations on the M2 proton channel of influenza A virus (Pielak et al., 2009; Schnell and Chou, 2008). They can also provide useful insights to stimulate drug developments as demonstrated by previous investigators (Cai et al., 2011; Chou, 2004b; Chou et al., 2003; Du et al., 2009a, 2010, 2005b; Huang et al., 2008; Li et al., 2011; Ma et al., 2012; Wang and Chou, 2011, 2012; Wang et al., 2009). The information of a binding pocket of a receptor for its ligand is very important for drug design, particularly for conducting mutagenesis studies (Chou, 2004b). In the literature, the binding pocket of a protein receptor to a ligand is usually defined by those residues that have at least one heavy atom (i.e., an atom other than hydrogen) within a distance of 5 Å from a heavy atom of the ligand. Such a criterion was originally used to define the binding pocket of ATP in the Cdk5-Nck5a* complex (Chou et al., 1999) that has later proved quite useful in identifying functional domains and stimulating the relevant truncation experiments (Zhang et al., 2002). The similar approach

has also been used to define the binding pockets of many other receptor–ligand interactions important for drug design (Chou, 2004a; Chou et al., 2003; Huang et al., 2008; Li et al., 2011; Pielak et al., 2009; Wang and Chou, 2011, 2012; Wang et al., 2007). Many marvelous biological functions in proteins and DNA and their profound dynamic mechanisms, such as switch between active and inactive states (Wang and Chou, 2009), cooperative effects (Chou, 1989), allosteric transition (Chou, 1987), intercalation of drugs into DNA [30], and assembly of microtubules (Chou et al., 1994), can be revealed by studying their internal motions as summarized in a comprehensive review (Chou, 1988). Likewise, to really understand the interaction of a protein receptor with its ligand and to reveal their

binding mechanism, we should consider not only the static structures concerned but also the dynamical information obtained by simulating their internal motions or dynamic process, and we are to make efforts in this regard in our future work. Virtual screening is a computational drug discovery method where large libraries of compounds are assessed for their potential to bind specific sites on drug target and identify small numbers of virtual hits (Klebe, 2006). Herein, virtual screening was performed by the pharmacophore model as ligand-based and molecular docking as structure-based screening. It is reported that about fifty percent of all drugs fail in coming to marketing stage because of poor human pharmacokinetics (Shen et al., 2003; Zheng and Tropsha, 2000). Thus,

Table 1a pIC₅₀ values for compounds of the general structure I.



Cmpd no.	R	R ₁	pIC ₅₀
1 ^a	H	H	5.00
2 ^a	4-Cl	H	4.70
3	4-F	4-Br	5.00
4	4-F	4-OCH ₃	5.00
5 ^a	4-OCH ₃	4-OCH ₃	5.30
6	4-OCH ₃	2,4-(OCH ₃) ₂	4.82
7	4-OCH ₃	2,6-(OCH ₃) ₂	6.43
8	4-OCH ₃	2,4,6-(OCH ₃) ₃	6.70
9	4-OCH ₃	3,4,5-(OCH ₃) ₃	4.46
10	2,4,6-(OCH ₃) ₃	4-OCH ₃	5.12
11	4-OCH ₃	2,6-(OCH ₃) ₂ , 4-OH	5.00
12	4-OCH ₃	2,4,6-F ₃	4.12
13 ^a	3-F, 4-OCH ₃	2,4,6-(OCH ₃) ₃	7.52
14	3-OCOCH ₂ C(CH ₃) ₂ -C ₆ H(CH ₃) ₂ O ₂ , 4-OCH ₃	2,4,6-(OCH ₃) ₃	7.40
15	3-OPO(ONa) ₂ , 4-OCH ₃	2,4,6-(OCH ₃) ₃	5.60
16	3-NH ₂ , 4-OCH ₃	2,4,6-(OCH ₃) ₃	4.12
17	3-NO ₂ , 4-OCH ₃	3,4,5-(OCH ₃) ₃	4.46
18	3-NH ₂ , 4-OCH ₃	3,4,5-(OCH ₃) ₃	4.00
19	3-NO ₂ , 4-OCH ₃	2,6-(OCH ₃) ₂ , 4-O(CH ₂) ₃ COOH	5.00
20	3-NH ₂ , 4-OCH ₃	2,6-(OCH ₃) ₂ , 4-O(CH ₂) ₃ COOH	5.00
21	3-OH, 4-OCH ₃	2,6-(OCH ₃) ₂ , 4-O(CH ₂) ₃ COOH	4.00
22	3-NO ₂ , 4-F	2,4,6-(OCH ₃) ₃	5.00
23	3-NH ₂ , 4-F	2,4,6-(OCH ₃) ₃	5.00
24	3,5-(NO ₂) ₂ , 4-OCH ₃	2,4,6-(OCH ₃) ₃	5.00
25	3,5-(NH ₂) ₂ , 4-OCH ₃	2,4,6-(OCH ₃) ₃	5.60
26 ^a	3-F, 4-OCH ₃	4-OCH ₃	5.30
27	3-F, 4-OCH ₃	2,3,4,5,6-F ₅	5.00
28	3-NO ₂ , 4-OCH ₃	2,3,4,5,6-F ₅	4.00
29	3-NH ₂ , 4-OCH ₃	2,3,4,5,6-F ₅	4.12
30	2,3,4,5,6-F ₅	3-NO ₂ , 4-OCH ₃	4.00
31	2,3,4,5,6-F ₅	3-NH ₂ , 4-OCH ₃	4.46
32	2,3,4,5,6-F ₅	2,3,4,5,6-F ₅	4.46

^a Prediction set.

in silico ADMET studies were performed on new compounds recognized by virtual screening to compare the computed ADMET descriptor values with the accepted ranges.

2. Materials and methods

2.1. Dataset

A set of 45 (E)-N-Aryl-2-ethene-sulfonamide analogues as anti-prostate cancer agents with associated activity data were collected from the literature (Ramana Reddy et al., 2013). The IC_{50} (μM) values of original paper were converted into molar units. These values were transformed into negative logarithm scale value (pIC_{50}) as dependent variable for all models subsequently developed. The pIC_{50} values of the data set extend from 4 to 7.5. For developing models, the total

compounds as usual were divided into two training and test sets consisting of 37 and 8 compounds, respectively. The chemical structures and experimental pIC_{50} values of all compounds are listed in Tables 1a–1c.

2.2. CoMFA and CoMSIA studies

The CoMFA and CoMSIA models were developed by SYBYL7.3 software package (Tripos Inc., St. Louis, USA, version 7.2) the details of model building steps can be found in the literature (Ghasemi and Shiri, 2012; Pirhadi and Ghasemi, 2010).

2.3. AutoGPA studies

To use the new alignment for the construction of a better model, AutoGPA software based on pharmacophoric

Table 1b pIC_{50} values for compounds of the general structure II.

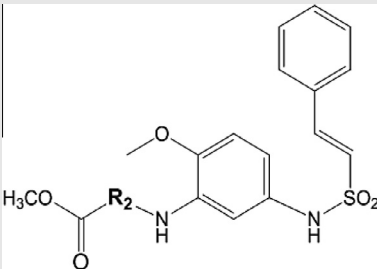
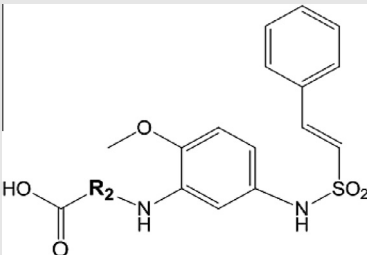
		
Cmpd no.	R ₂	pIC_{50}
33	CH ₂	6.46
34	CH(CH ₃)	7.00
35	C(CH ₃) ₂	6.70
36 ^a	CH(C ₆ H ₅)	5.60
37	CH(C ₆ H ₄ -F)	5.60
38	CH(C ₆ H ₄ -Br)	5.12

Table 1c pIC_{50} values for analogous of the general structure III.

		
Cmpd no.	R ₂	pIC_{50}
39	CH ₂	6.40
40	CH(CH ₃)	7.40
41	C(CH ₃) ₂	7.15
42	CH(C ₆ H ₅)	7.12
43 ^a	CH(C ₆ H ₄ -F)	7.12
44	CH(C ₆ H ₄ -Cl)	7.12
45 ^a	CH(C ₆ H ₄ -Br)	6.60

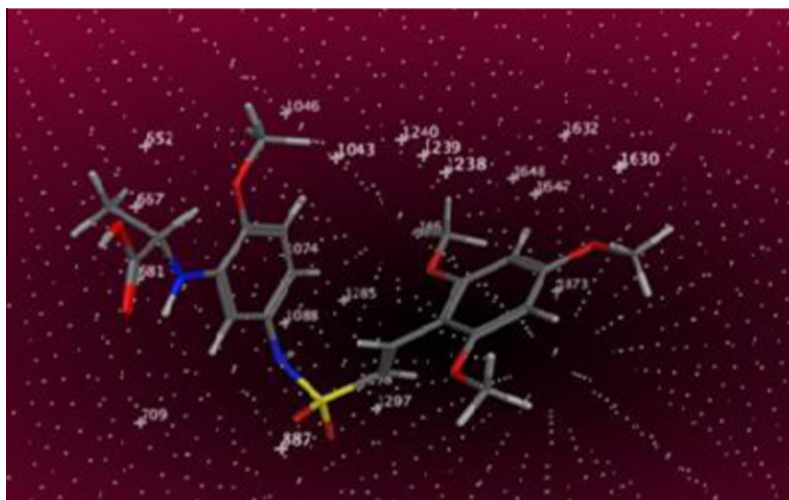


Figure 2 Important steric and electrostatic fields around the aligned compounds generated by AutoGPA model. For better presentation, only one molecule was shown instead of the superposition of all molecules.

alignment which is a grid potential analysis that evaluates steric and electrostatic fields around pharmacophore-based aligned ligands, has been used, Fig. 2 (Asakawa et al., 2012). MMFF94s force field with generalized Born solvation model was employed for energy minimization of structures. Two separate databases that consist of structures of molecules and their related pIC_{50} , were created for training and test sets. Conformation search option in MOE2009.10 was used to generate probable conformers for each molecule in data sets. Strain energy for conformations was considered less than 7 kcal/mol and RMSD gradient was defined 2.5 Å by default. Conformer generation was continued until a maximum number of 10,000 conformers per ligand (Chen and Foloppe, 2008). Finally, 38,837 conformations for training set and 3784 conformations for test set were generated.

2.3.1. Pharmacophore elucidation and alignment

The generated conformers for ligands should be aligned over the common pharmacophore queries. Hydrogen bond acceptor, hydrogen bond donor, hydrophobic area and positively and negatively ionized areas as pharmacophore features were searched in each conformation. When biologically active molecules join to binding site of receptor, they form similar interactions, so, it is expected that their bioactive conformation carries common three-dimensional arrangement of pharmacophores. The option named AutoGPA looked for pharmacophore queries (three-dimensional arrangement of the pharmacophore features) that derive good overlay of the most active molecules. At last, using this option, pharmacophore queries which were satisfied by all or a large number of the most active molecules were extracted and used to align all compounds of training set. Common geometry features of active compounds in case no 3D structure of target is available, can give information about key interactions between the active molecules and receptor (Asakawa et al., 2012; Kumar and Tiwari, 2013).

2.3.2. 3D-QSAR model construction

AutoGPA-based 3D-QSAR model was generated by superimposed ligands using pharmacophore alignment. It calculates

fields based on theory of CoMFA. A cubic lattice with 2.0 Å grid spacing surrounding all of the compounds was created and a probe atom that was sp^3 carbon atom with charge +1.0 was used at each intersection grid point. Steric and electrostatic energies interaction based on van der Waals and coulombic between atoms and probe was calculated (Pirhadi and Ghasemi, 2010). In calculation of grid potential, 30 kcal/moles limit of van der Waals potential and a threshold 0.15 for maximum variance were considered. PLS regression was used to develop a model that correlates grid potential fields to biological activity. The optimum number of components was fixed to 10. In this manner, 3D-QSAR models were constructed, sorted based on q^2 criterion of leave-one-out crossvalidation (LOOCV) (Section 2.4) and final AutoGPA model was achieved.

2.4. Validation of models

Crossvalidation as an internal validation technique was used to assess the prediction quality of the models. In a standard internal validation method, named LOOCV (leave one out crossvalidation), one compound is excluded from the original training set, and a new model is built based on new training set and this model is used to predict the activity of the excluded one. For each model, this procedure is repeated for whole compounds of data set, and each compound has been excluded once, then cross validated q^2 which is considered as a criterion of robustness and predictive ability of the models, was calculated by Eq. (2) (Cramer et al., 1988a; Wold, 1978) as follows:

$$q^2 = 1 - \frac{\sum (y_i - \hat{y})^2}{\sum (y_i - \bar{y})^2} \quad (1)$$

where \bar{y} presents average activity value of the entire dataset and y_i and \hat{y} are observed and predicted activity values, respectively. A high q^2 value ($q^2 > 0.5$) is used as an evidence of high predictive ability of the model (Hawkins et al., 2003). Golbarikh and Tropsha reported that the high value of q^2 is essential and important but not adequate for a predictive model (Lu et al., 2010) and an external analysis by test set of molecules should be employed to investigate the prediction of model.

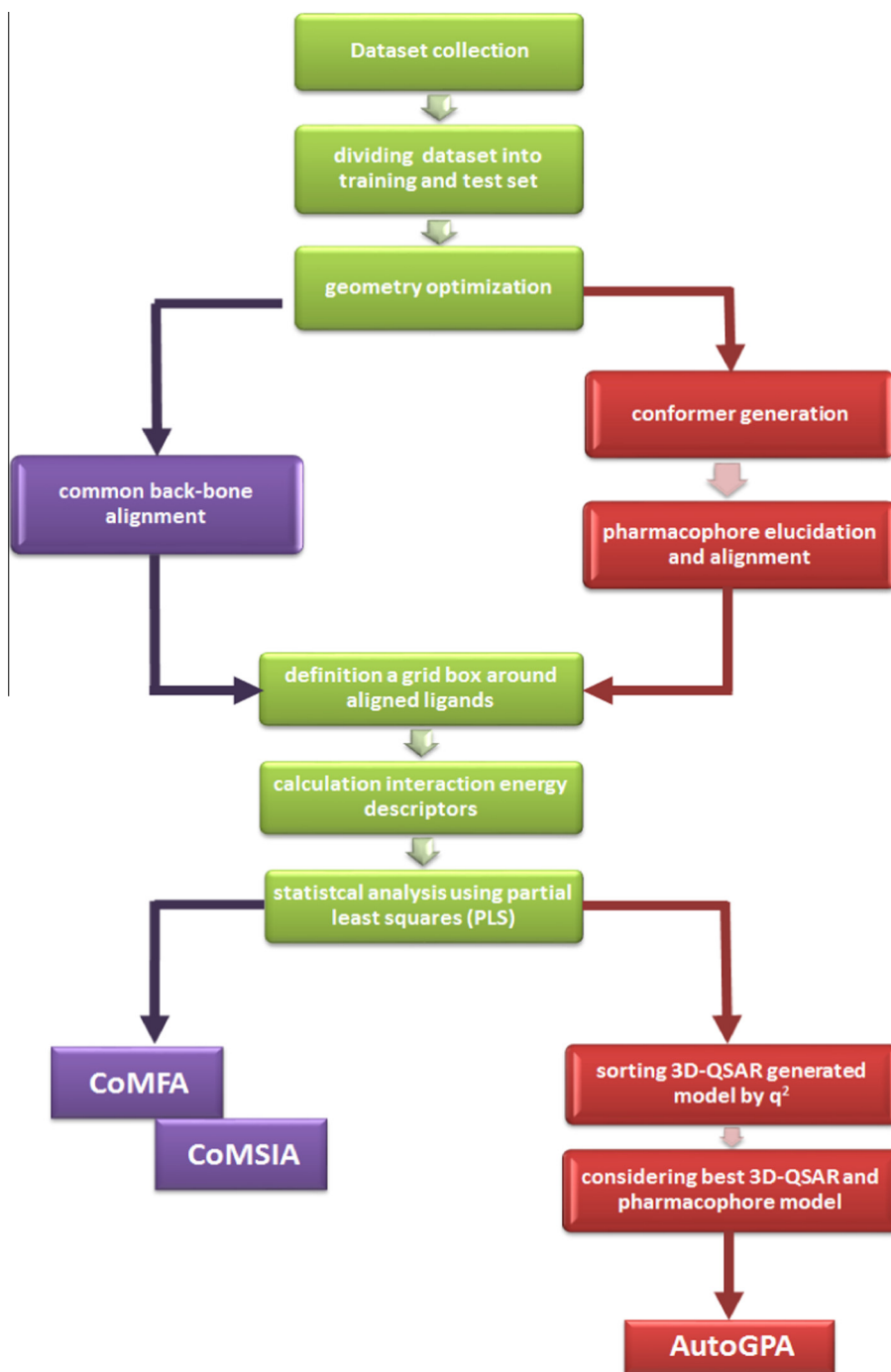


Figure 3 Workflow for the process of generation of AutoGPA, CoMFA and CoMSIA, in this study.

They said a QSAR model is predictive if it fulfills the following conditions (Tropsha et al., 2003):

$$q^2 > 0.5$$

$$R^2 > 0.6$$

$$\frac{(R^2 - R_0^2)}{R^2} < 0.1 \text{ or } \frac{(R^2 - R_0'^2)}{R^2} < 0.1$$

$$0.85 \leq k \leq 1.15 \text{ or } 0.85 \leq k' \leq 1.15$$

where R^2 is squared correlation coefficient values between the observed and predicted values of the test set compounds (Lu

et al., 2010). The R_{pred}^2 value was calculated according to Eq. (2):

$$R_{\text{pred}}^2 = 1 - \frac{\sum_{i=1}^m (y_i - \hat{y}_i)^2}{\sum_{i=1}^m (y_i - \bar{y})^2} \quad (2)$$

where \hat{y}_i and y_i indicate predicted and observed activity values of the test set, respectively, and \bar{y} is the average value of training set activities and m is the number of compounds in the test set (Ebrahimi et al., 2013). R_0^2 and $R_0'^2$ are squared correlation coefficient values for observed versus predicted and predicted

versus observed activities, through origin, respectively and k' is the slope of regression lines through the origin (Lu et al., 2010).

In addition, for better predictive potential of models, Roy and Roy (2008) introduced following modified r^2 (r_m^2):

$$r_m^2 = r^2 \left(1 - \left| \sqrt{r^2 - r_0^2} \right| \right) \quad (3)$$

If r_m^2 value for a model is greater than 0.5, it states high predictive power of model. The concept of r_m^2 was applied to the prediction of test set [r_m^2 (test)] and overall set of compounds [r_m^2 (overall)] (Pratim Roy et al., 2009).

To demonstrate the reliability of predictions of AutoGPA model, applicability domain was determined by leverage approach (Dragos et al., 2009). Standardized residuals versus leverage values visualize the applicability domain of the model. The warning leverage is calculated as $3(k + 1)/n$, where k is the number of latent variables of the model and n is the number of training set molecules. Compounds with higher leverage value than h^* are called X outliers that indicate structurally significant chemicals, and compounds which have higher standardized residual than 3 are considered as Y outliers (Stanforth et al., 2007; Tetko et al., 2008).

2.5. Molecular docking

X-ray structure of tubulin with the stoichiometry “hetero-5-mer-A2B2C” was extracted from protein data bank (PDB code: 3HKE). Docking studies were conducted on colchicine as one of binding pockets of tubulin by Gold algorithm using Discovery Studio 2.5 (Discovery Studio). Ligand structures were typed with CHARMM force field and partial charges

were calculated by Momany-Rone option. Then, resulting structures were minimized with Smart Minimizer which performs 1000 steps of steepest descent with a RMS gradient tolerance of 3, followed by conjugate gradient minimization. In next step, for preparation of tubulin, complex typed with CHARMM force field, hydrogen atoms were added, all water molecules were removed and pH adjusted to neutral 7.4. A 8 Å radius sphere was defined as the binding region for docking study. Other parameters were set by default protocol settings. Gold method employs a strong genetic algorithm approach for conformational search and molecular docking (Politi et al., 2010) to guess, how fine the compounds join to the tubulin active site (Sakkiah et al., 2011). This program considers complete flexibility of side chains of residues of receptor at binding site. Finally, all of the datasets were docked into binding site of receptor, and fitness scores were obtained. Compounds with larger fitness score have been docked better (Ardakani and Ghasemi, 2013; Li et al., 2012).

2.6. Virtual screening and ADMET analysis

The ZINC database was screened with the best generated pharmacophore AutoGPA model through ZINCPharmer (<http://ZINCpharmer.csb.pitt.edu>) (Jasuja et al., 2014). ZINCPharmer is an online website that uses pharmer pharmacophore search method to explore ZINC database (Koes and Camacho, 2012). Molecular weight (< 500), total number of rotatable bonds to 10 and number of conformations per molecule to 1 were considered as primary filters in this step of screening. Then, the obtained hits were filtered using Lipinski's rules (Ca, 2000; Lipinski CAF and Feeney, 1997; Yadav and Khan, 2013). The remaining molecules were screened by

Table 2 Summary of the results obtained from the CoMFA, CoMSIA and AutoGPA analyses.

Component	CoMFA	CoMSIA	AutoGPA
q^2	0.69	0.56	0.55
R_{ncv}^2	0.86	0.75	0.92
RMSEC	0.4	0.41	0.35
n	5	3	3
RMSEP	1.07	0.85	0.42
F value	53.25	32.40	206.04
R_{pred}^2	0.68	0.76	0.91
$(R^2 - R_0^2)/R^2$	0.08	0.08	0.06
k	0.84	0.88	0.98
k'	1.17	1.12	1.02
r_m^2 (overall)	0.68	0.79	0.89
r_m^2 (test)	0.52	0.53	0.65
Fraction			
Steric	0.88	0.17	0.11
Electrostatic	0.12	0.05	0.89
Hydrophobic		0.32	
H-bond donor		0.26	
H-bond acceptor		0.2	

q^2 = Cross-validated correlation coefficient after the leave-one-out procedure.

R_{ncv}^2 = Non-cross-validated correlation coefficient.

RMSEC = Root-mean-square-error for training set.

n = optimum number of components.

RMSEP = Root-mean-square-error for test set.

R_{pred}^2 = predictive correlation coefficient.

R_0^2 = correlation between predicted versus observed activity setting the intercept zero.

r_m^2 = modified squared correlation coefficient.

docked into binding site of receptor by Gold algorithm. Absorption, distribution, metabolism and excretion, ADME parameters, were calculated to investigate pharmacokinetic properties of hit compounds. If a drug absorbed fast and completely by gastrointestinal tract (GIT), distributed selectivity to its receptor, its effect did not eliminate quickly in metabolization and in excretion process, removed in a proper procedure without creating any harm, is considered ideal. Some chemical descriptors were used to calculate pharmacokinetics properties that have relationship with chemical structure (Hodgson, 2001; Winiwarter et al., 1998). ADME studies on virtual hits were performed by Qikprop version 3.2 of Schrodinger suite, (2010) (Schrodinger Suite, 2010: QikProp and New York).

To assess toxicity risk for obtained hits, mutagenicity, tumorigenicity, irritant and reproductive effects were investigated by OSIRIS property explorer (Explorer. et al.). Also, drug likeness and drug score values were determined for each compound.

3. Results and discussion

3.1. Comparison of 3D-QSAR models

The 3D-QSAR models were derived for a dataset of 45 tubulin inhibitors. Fig. 3 shows the trend followed for the development

Table 3 The experimental and predicted pIC50 values of all compounds.

Compound no.	Experimental	CoMFA		CoMSIA		AutoGPA	
		Pred.	Res.	Pred.	Res.	Pred.	Res.
1 ^a	5	4.88	0.12	4.90	0.1	5.31	-0.31
2 ^a	4.7	4.67	0.03	4.94	-0.24	5.12	-0.42
3	5	4.84	0.16	4.67	0.33	4.65	0.35
4	5	4.91	0.09	4.75	0.25	4.66	0.34
5 ^a	5.3	4.56	0.74	4.58	0.72	5.51	-0.21
6	4.82	4.93	-0.11	5.14	-0.32	4.88	-0.06
7	6.43	4.96	1.47	5.50	0.93	6.29	0.14
8	6.7	5.81	0.89	5.58	1.12	6.73	-0.03
9	4.56	4.71	-0.25	5.00	-0.54	4.45	0.01
10	5.12	5.26	-0.14	4.79	0.33	5.1	0.02
11	5	5.78	-0.78	5.38	-0.38	5.53	-0.53
12	4.12	4.49	-0.37	4.56	-0.44	5	-0.88
13 ^a	7.52	5.7	1.82	5.82	1.7	6.68	0.84
14	7.4	7.41	-0.01	7.48 47	-0.08	6.77	0.63
15	5.6	5.6	0.00	5.61	-0.01	5.48	0.12
16	4.12	5.1	-0.98	4.97	-0.85	5.27	-1.15
17	4.46	4.35	0.11	4.52	-0.06	4.45	0.01
18	4	4.07	-0.07	4.33	-0.33	4.34	-0.34
19	5	4.78	0.22	4.58	0.42	5.22	-0.22
20	5	4.87	0.13	4.54	0.46	5.17	-0.17
21	4	4.35	-0.35	5.07	-1.07	4.3	-0.3
22	5	4.85	0.15	5.05	-0.05	5.09	-0.09
23	5	4.92	0.08	5.03	-0.03	5.01	-0.01
24	5	5.13	-0.13	5.02	-0.02	5.17	-0.17
25	5.6	5.08	0.52	5.33	-0.27	5.93	-0.33
26 ^a	5.3	4.22	1.08	4.59	0.71	5.24	0.06
27	5	4.73	0.27	4.79	0.21	5.12	-0.12
28	4	3.9	0.10	4.12	-0.12	4.43	-0.43
29	4.12	3.82	0.30	4.05	0.07	4.4	-0.28
30	4	4.32	-0.32	3.86	0.14	3.56	0.44
31	4.46	4.37	0.09	4.30	0.16	4.38	0.08
32	4.46	4.55	-0.09	4.67	-0.21	4.04	0.42
33	6.46	6.62	-0.16	6.63	-0.17	6.47	-0.01
34	7	7	0.00	6.56	0.44	6.8	0.2
35	6.7	6.86	-0.16	6.86	-0.16	6.87	-0.17
36 ^a	5.6	5.33	0.27	5.42	0.18	5.4	0.2
37	5.6	5.33	0.27	5.52	0.08	5.4	0.2
38	5.12	5.18	-0.06	5.27	-0.15	5.43	-0.31
39	6.4	6.35	0.05	6.83	-0.43	6.42	-0.02
40	7.4	7.37	0.03	7.25	0.15	7.34	0.06
41	7.15	6.83	0.32	7.35	-0.2	7.35	-0.2
42	7.12	7.32	-0.2	6.97	0.15	7.1	0.02
43 ^a	7.12	5.53	1.59	6.03	1.09	6.55	0.57
44	7.12	7.22	-0.1	7.04	0.08	6.7	0.42
45 ^a	6.6	5.4	1.20	5.81	0.79	6.66	-0.06

^a Prediction set.

of CoMFA, CoMSIA and AutoGPA models. The statistical parameters associated with the models were listed in Table 2. Although results indicate the CoMFA model gives higher q^2 than those obtained by AutoGPA and CoMSIA, other specifications of AutoGPA model are better. The prediction ability of models was determined using a set of 8 test compounds not included in the model generation. As we can see in this table, non-cross-validated correlation coefficients and the predicted R^2_{pred} value of AutoGPA model were 0.92 and 0.91 with a number of optimized components of 3. Therefore, this model can be used reliably in new inhibitors design. The values of experimental and predicted activities of all models are depicted in Table 3. The graphs of actual activity versus predicted activity of the training set and test set for all models are illustrated in Fig. 4a–c. The analysis of the applicability domain of the AutoGPA model, Fig. 5, denotes no outliers in the test set and demonstrates the ability of model in predicting the action of new compounds.

3.2. Contour maps analysis

To visualize the information of derived models contour maps are generated. They are calculated as the product of the standard deviation of field (StDev) at each grid point and PLS

coefficient. The contour maps show regions where differences in molecular fields are related to differences in activity (Mao et al., 2012). Interpretation of the steric and electrostatic contours, using compound 40 as the template structure of AutoGPA model is trivial and displayed in Fig. 6a and b. The steric maps are represented by green and yellow contours while the green color indicates regions in space around aligned molecules where bulky groups would favorably interact with the receptor and yellow color shows regions where bulky groups would decrease the activity. Fig. 6a shows the yellow region near 6-methoxy (R_1) substituent that suggests a bulky group is not favorable and decreases the activity. It can explain the activity of compound 16 lower than compound 17 due to the existence of OCH_3 bulky group. A small green contour around position 3rd of terminal phenyl ring (R) reveals the bulky substituent increases the activity. For example, the comparison of this position in compounds 14 ($\text{OCOCH}_2\text{C}(\text{CH}_3)_2\text{C}_6\text{H}$) with 8 (without any substituent), 15 ($\text{OPO}(\text{ONa})_2$) and 16 (NH_2) confirms the observation. The electrostatic field represented by blue contours shows regions where an electron donating group would increase the activity and red contours indicate regions where electron withdrawing group would increase the activity. A small red region near position 5th (R_1) shows the electronegative substituent causes increasing of the activity (Fig. 6b). The compound 9 with electron donating group $-\text{OCH}_3$ is less activate in comparison with compound 8. In the same way, the activity of compound 16 (without any substituent) is more than compound 18 (OCH_3). Two blue regions near 4-methoxy group in positions R_1 and R indicate electron donating groups would increase the activity demonstrated by the order of activities in some compounds such as: $8 > 7$, compound 8 has an extra OCH_3 at R_1 ; $5 > 4$, compound 5 possesses an OCH_3 instead F in compound 4; and $1 > 2$, compound 2 with Cl at R indicates lower activity than compound 1 with H atom. The big blue contour near side chain R_2 exhibits the electron withdrawing group decreases biological activity. Taking the compounds 36–38 ($R_2 = \text{CH}(\text{C}_6\text{H}_5)$, $\text{CH}(\text{C}_6\text{H}_4\text{-F})$, $\text{CH}(\text{C}_6\text{H}_4\text{-Br})$), the electron withdrawing groups at the R_2 situation resulted in decreasing activity with regard to compounds 34 and 35 ($R_2 = \text{CH}(\text{CH}_3)$, $\text{C}(\text{CH}_3)_2$). Fig. 7a provides the hydrophobic contour for compound 40 of the CoMSIA model.

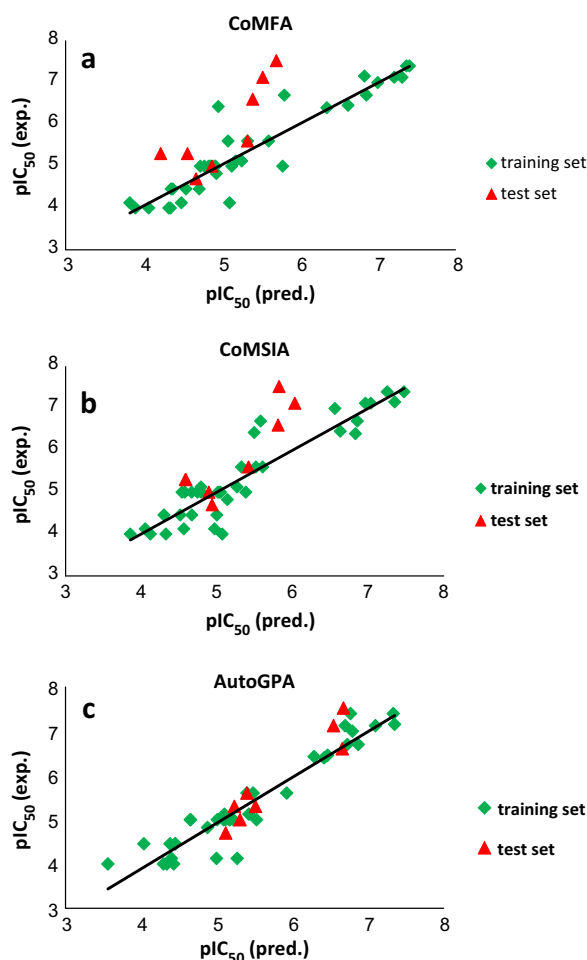


Figure 4 Observed vs. predicted activities for the training and test sets of compounds based on CoMFA (a), CoMSIA (b) and AutoGPA (c) models.

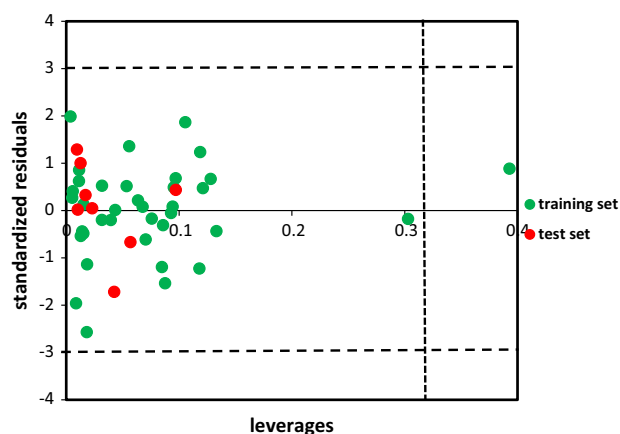


Figure 5 Standardized residuals vs. leverages. Dashed lines represent ± 3 standardized residual and dotted line represents warning leverage ($h^* \approx 0.32$).

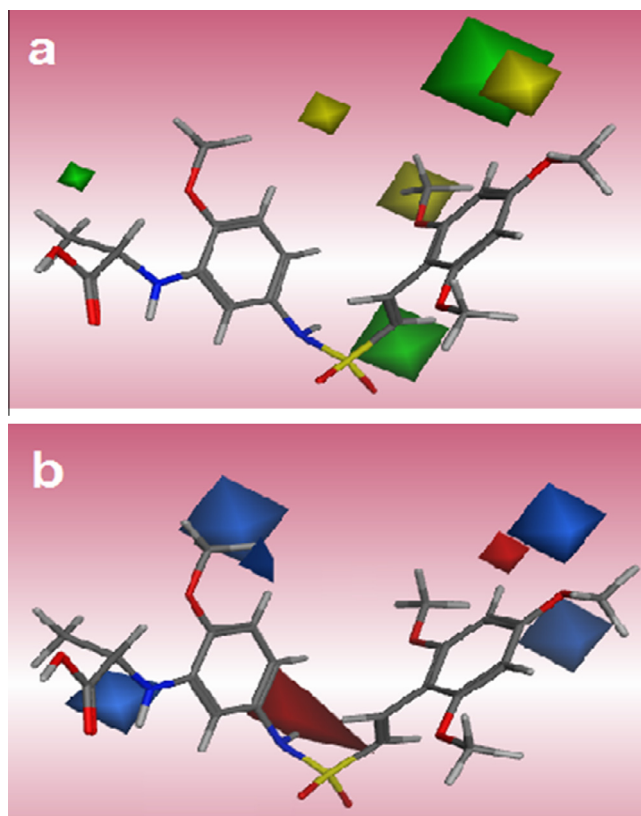


Figure 6 AutoGPA contour maps based on compound 40: steric (a), electrostatic (b).

The yellow and white plots indicate hydrophobicity and hydrophilicity in that region is expected to increase the activity of the molecule. In Fig. 7a, two yellow contours around 4-methoxy group at R_1 and R positions which favored the existence of hydrophobic substituents are toward these regions. Compound 8 with hydrophobic group (OCH_3) in 4 th situation of R_1 displayed higher activity than compound 12 (F). One white contour near amine group in 3 rd situation of R suggests that a hydrophilic group may be favored. This can explain why derivative 25 exhibited higher activity than compound 24 (compound 25 possesses an NH_2 instead NO_2 in compound 24) and also compound 29 exhibited higher activity than 28 (compound 29 possess an NH_2 instead NO_2 in compound 28). The hydrogen bond donor field interaction for CoMSIA model was shown in Fig. 7b. The cyan contours display the hydrogen bond donor groups are favorable while purple contours state these groups would decrease activity. One medium size cyan contour around 4-methoxy (R_1) suggests that hydrogen donor group in this region would increase activity. This is a possible reason why compound 11 has higher activity than compound 2 and also compounds 19 and 20 have higher activity than compounds 16, 17 and 18. Two purple contours near 2- and 5-methoxy positions of R_1 indicated that hydrogen bond acceptor substituent would benefit the activity. This may explain why derivative 32 with two hydrogen bond acceptor substituents (-F) at these positions exhibits higher activity than compound 30 (without any substituent). In hydrogen bond acceptor field, the magenta and red plots indicate favorable and unfavorable situations. In Fig. 7c, one H-bond acceptor favorable to magenta contour located near 6-methoxy (R_1)

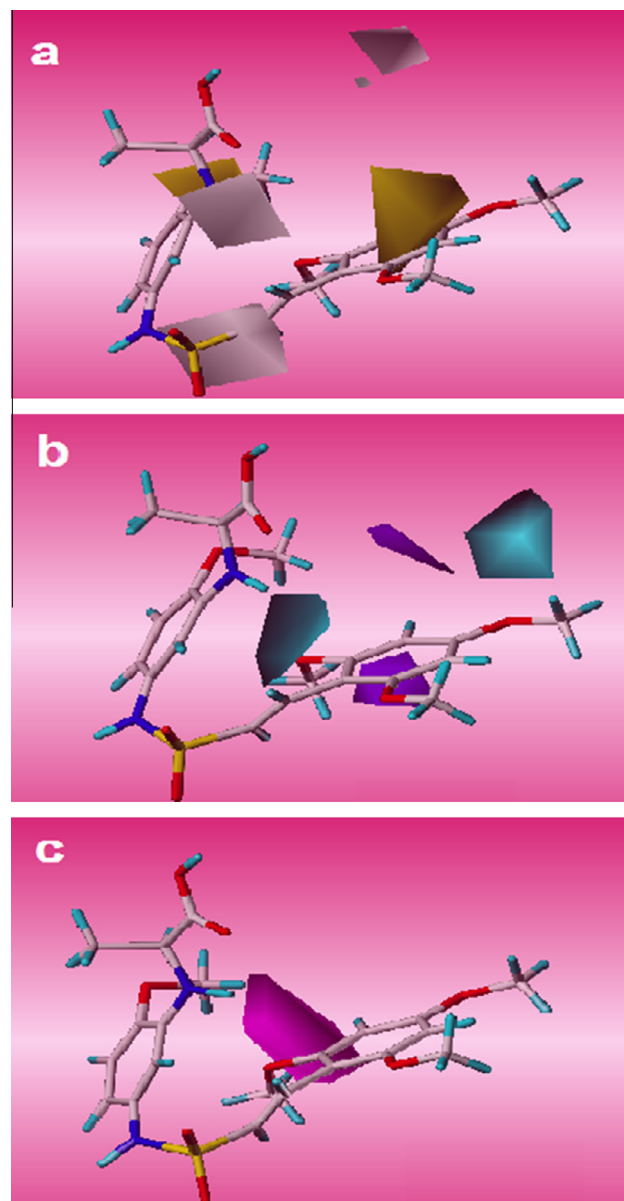


Figure 7 CoMSIA contour maps based on compound 40: hydrophobic (a), hydrogen donor (b) and hydrogen bond acceptor (c).

considering cyan contour of hydrogen donor field in this situation, revealed that both hydrogen bond donor and acceptor groups can increase activity. Great number of the derivatives involved in this study possessed hydrogen bond acceptor group (OCH_3) at this site which demonstrates the importance of the hydrogen bond acceptor substituent. Fig. 8 indicates the best pharmacophore hypothesis including 4 features. An orange sphere as aromatic (Aro|PIR) π -ring center on the phenyl ring states all compounds can create π -interaction from this position. Three blue spheres oriented toward sulfur monoxide of all compound, amine group and methoxy group in 3 rd and 4 th situations of R (Acc2), respectively. This feature shows the ability of diverse positions of compounds of data set for establishing hydrogen bond. The common pharmacophoric features can help in screening out the potential ligand. Modification of substituents of the phenyl rings considering the

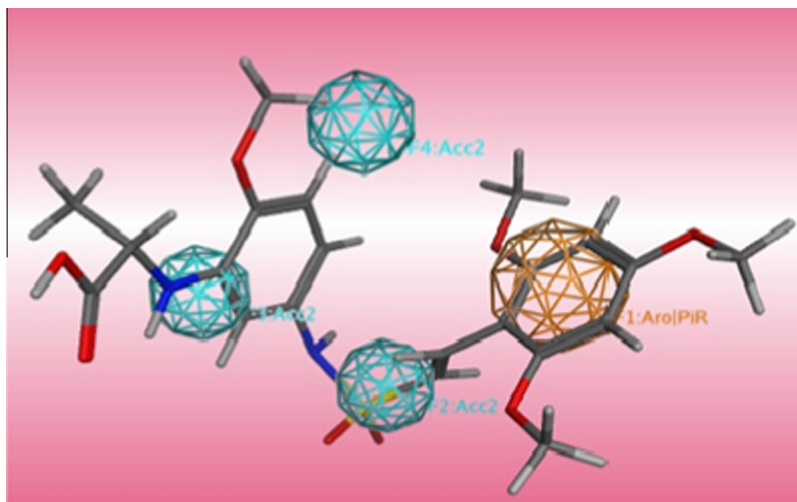


Figure 8 The best pharmacophore model of AutoGPA, mapping with the most active compound 40.

derived AutoGPA models (3D-QSAR and pharmacophore) motivated us to propose more desirable compounds.

3.3. Molecular docking analysis

Molecular docking was employed to find binding mode of compounds, and additionally to evaluate the reliability of obtained contour maps and pharmacophore model. All compounds were docked in colchicine site of target. The most active compounds in dataset 14 and 40 were selected for more detailed analysis. Fig. 9 displays the hydrogen bonds between the compound 40 and the binding site of receptor, the key residues were labeled. Investigation of the binding mode of compound 40 shows a hydrogen bond between O of 6-methoxy (R_1) and Thr179 from the α chain as is mapped to the magenta contour in CoMSIA. In addition, there is one hydrogen bond between O of carbonyl group 3rd situation of R with Val181 from the same chain. The 4-methoxy (R_1) formed H-bond with Ala250 from the β chain of tubulin. The hydrogen atoms of amine group of the molecular frame and hydroxyl group on substituent 3 (R) act as hydrogen bond donor against Ser178 from the α chain and Asn349 from the β chain, respectively. In compound 14, carbonyl group 3rd situation of R as hydrogen bond acceptor gives a hydrogen bond with nitrogen atom of amino acid Thr353. Meanwhile, there are not any hydrogen

bonds in inactive compound 28, and it could confirm what is the reason of weaker binding between inactive compounds and the binding site. There is an π -cation interaction between the conjugated ring on 3rd situation of R in compound 14 and ammonium group of Lys352 which confirms the ring aromatic feature of pharmacophore model. Docking results illustrated many electrostatic interactions between 2, 4 and 6-methoxy groups (R_1) with residues of binding pocket. 4-Methoxy group (R) of compound 14 has electrostatic interaction with polar residues Gln176, Ser178 and Thr179 from the α chain and Lys352 from the β chain in compliance with the blue contour map of AutoGPA model. Several polar residues such as Lys352, Thr314, Asn258 and 350 from the β chain around side chain R_2 of template confirm the increasing electropositivity of this substituent, and the more power interaction can be formed, that is in agreement with electrostatic blue contour map AutoGPA.

3.4. Virtual screening analysis

Fig. 10 shows summarized protocol for virtual screening of the molecular libraries in this study. The pharmacophore model usually gives an accurate hypothesis of required attributes for a new lead (Gopalakrishnan et al., 2005). The optimized derived AutoGPA model was used as 3D virtual query to

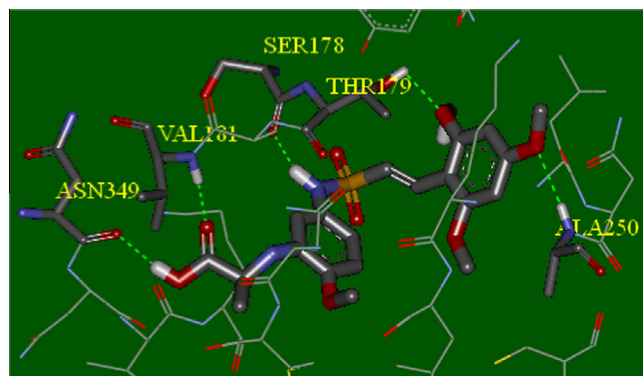


Figure 9 Hydrogen bonding interactions between compound 40 and key residues.

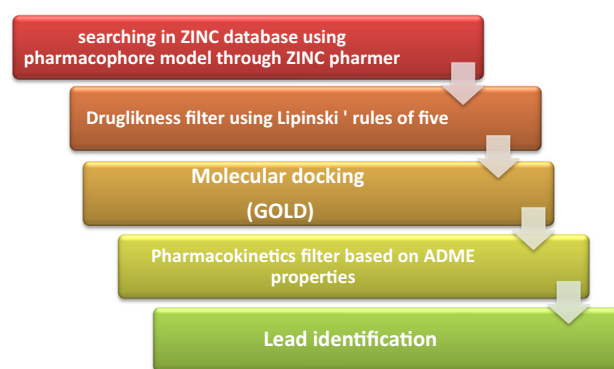


Figure 10 Workflow for combined ligand-based and structure-based virtual screening.

Table 4 Structure of retrieved compounds from virtual screening with their Gold fitness scores.

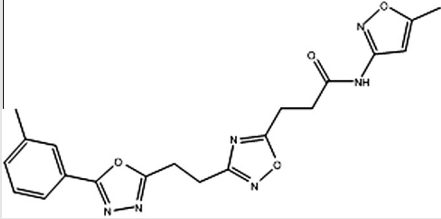
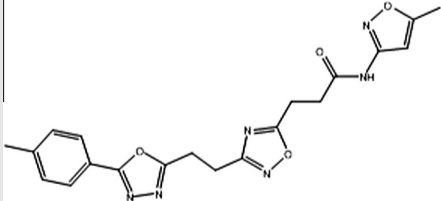
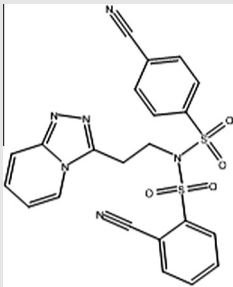
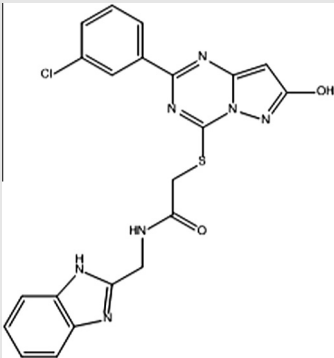
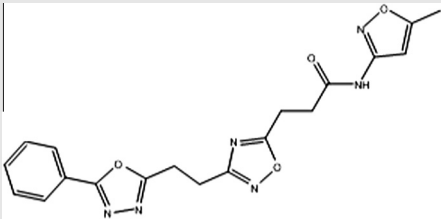
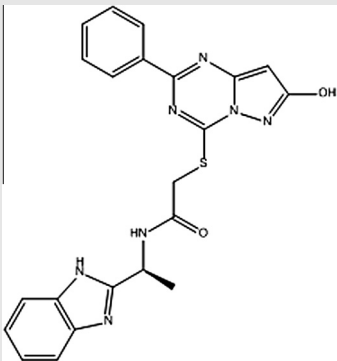
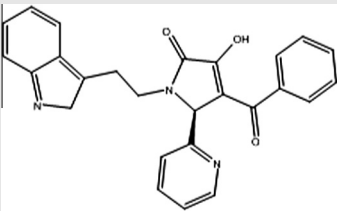
Compound name	Structure	ZINC number	Gold score
Vs1		ZINC21721788	72.78
Vs2		ZINC21721781	71.84
Vs3		ZINC12569439	77.65
Vs4		ZINC21537504	74.88
Vs5		ZINC21721756	72.20

Table 4 (continued)

Vs6		ZINC20987079	74.76
Vs7		ZINC63212443	73.96

screen the ZINC database for finding new and potent hits. 693 conformers were obtained from the initial screening. As the second step, Lipinski's rules assessed the drug-likeness of remained compounds, a subset of 488 molecules were retrieved. In the subsequent structure-based screening step, they were docked into active site of protein to refine the hits using defined sphere of compounds in the data set by the Gold program. Finally, 7 hits which were docked well and gave high Gold fitness scores, are listed in Table 4. Fitness scores were more than the most active compound of data set with score 52.77. The 7 hits fitted in 4 diverse structural scaffolds and were different from the compounds in the training data set. More investigation was performed on Vs3 with Gold fitness score of 77.65. Cyanide group attached to phenyl ring of this hit that mapped with acceptor feature in pharmacophore model acts as hydrogen bond acceptor and forms two hydrogen bonds with Ala20 and Asp251 residues. The methyl group in meta position of aromatic ring in compound Vs1 forms several hydrophobic interactions with Val238, Leu242 and 255 hydrophobic residues. The methyl group in para position

Vs2 is exposed to polar amino acids Asn258 and Thr239 and 40. So, this hit is unable to establish appropriate interaction in this position and shows the Gold fitness score less than Vs1. The results indicated the AutoGPA pharmacophore model is a powerful tool in finding new inhibitors for virtual screening.

3.5. ADMET studies

All the seven reported hits have drug-likeness properties according to Lipinski's rules. We investigated several physico-chemical properties related to pharmacokinetic parameters for these molecules and results are summarized in Table 5. Log *P* was calculated to measure the hydrophobicity of compounds. Compound with high lipophilicity and also high log *P* will have a poor surface contact with the gut and likely poor absorption. It is known that compounds with a log *P* < 5 can be absorbed well. Aqueous solubility (log *S*) was calculated to recognize poorly soluble compounds because a low solubility governed by log *S* causes a bad absorption, distribution and absorption

Table 5 Prediction of ADME properties of compounds obtained from virtual screening using Qikprop.

Descriptors	Vs1	Vs2	Vs3	Vs4	Vs5	Vs6	Vs7	Stand. range ^a
Apparent Caco-2 permeability (nm/s)	158	114	37	44	195	148	470	< 25 poor, > 500 great
Apparent MDCK permeability (nm/s)	158	81	14	139	153	146	218	< 25 poor, > 500 great
<i>J_m</i> (max. transdermal transport rate)	0.002	0.001	0.028	0.001	0.006	0.005	0.226	μg/cm ² h
Log <i>S</i> (aqueous solubility)	-4.615	-4.483	-3.532	-5.645	-4.601	-5.522	-4.278	-6.5/0.5
% human oral absorption in GI (± 20%)	80	76	62	77	81	85	96	< 25% is poor
log <i>BB</i> for brain/blood	-1.709	-1.840	-2.592	-2.065	-4.601	-1.701	-1.102	-3.0/1.2
log <i>K_{hsa}</i> (serum protein binding)	-0.125	-0.159	-0.975	0.249	-0.261	0.180	0.158	-1.5/1.5
log <i>P</i> for octanol/water	2.348	2.144	1.220	3.436	2.218	3.322	3.697	-2.0/6.5
Skin-permeability coefficient (log <i>K_p</i>)	-3.655	-3.999	-3.713	-3.289	-3.206	-2.388	-1.994	-8.0 to -1.0, <i>K_p</i> in cm/h

^a For 95% of known drugs, based on Qikprop v.3.2 (Schrödinger, USA, 2009) software results.

Table 6 Toxicity risk assessment test on (E)-N-Aryl-2-ethene-sulfonamide analogues.

S. no.	MUT	TUM	IRR	RE	CLP	S	DL	DS
Vs1	No risk	No risk	No risk	Medium risk	2.86	−5.67	1.07	0.38
Vs2	No risk	No risk	No risk	Medium risk	2.86	−5.67	−1.34	0.26
Vs3	No risk	No risk	No risk	No risk	1.12	−4.13	−3.34	0.33
Vs4	No risk	No risk	No risk	No risk	2.49	−6.06	4.87	0.40
Vs5	No risk	No risk	No risk	Medium risk	2.51	−5.32	1.67	0.44
Vs6	No risk	No risk	No risk	No risk	2.17	−5.39	6.66	0.57
Vs7	No risk	No risk	No risk	No risk	1.98	−3.07	5.99	0.78

MUT: Mutagenicity; TUM: Tumorigenicity; IRR: Irritating Effects; RE: Reproductive effects; CLP: cLogP; S: Solubility; DL: Drug likeness; DS: Drug Score (Calculated through OSIRIS property explorer).

of a compound. Calculations related to blood–brain barrier (logBB) and apparent MDCK cell permeability, gut–blood barrier (Caco-2 cell permeability), transdermal transport rate (J_m), aqueous solubility, % human oral absorption in GI ($\pm 20\%$) serum protein binding, log P for octanol/water and skin permeability (k_p) showed that these values for hits fell within the standard range of 95% of known drugs for drug likeness (Yadav and Khan, 2013). The results of toxicity risk assessment indicates that hits do not have any mutagenicity, tumorigenicity and irritant effects, and reproductive effects for 3 hits had medium limit. The calculated toxicity risk assessment parameters for virtually screened hits are listed in Table 6.

4. Conclusion

In this study, 3D QSAR modeling, molecular docking, virtual screening and ADMET approaches were employed to predict the biological activity of compounds and find the probable lead compounds from virtual screening. The CoMFA and CoMSIA models were generated using common substructure-based alignment of compounds in training set. The AutoGPA model as a pharmacophore alignment-based method was applied to find the bioactive conformers and yielded better predictive and reliable model. The calculation of parameters for prediction ability of the derived models confirms that the AutoGPA model is more predictive. Also, the AutoGPA contour maps were more interpretable to explain the effective features on activity. The pharmacophoric model, Lipinski's rules and molecular docking screened ZINC database to suggest 7 hits with high score fitness and diverse structures. *In silico* ADMET studies indicated the hits are in standard ranges. Therefore, AutoGPA model can be used as a beneficial method for screening large library and finding new inhibitors.

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